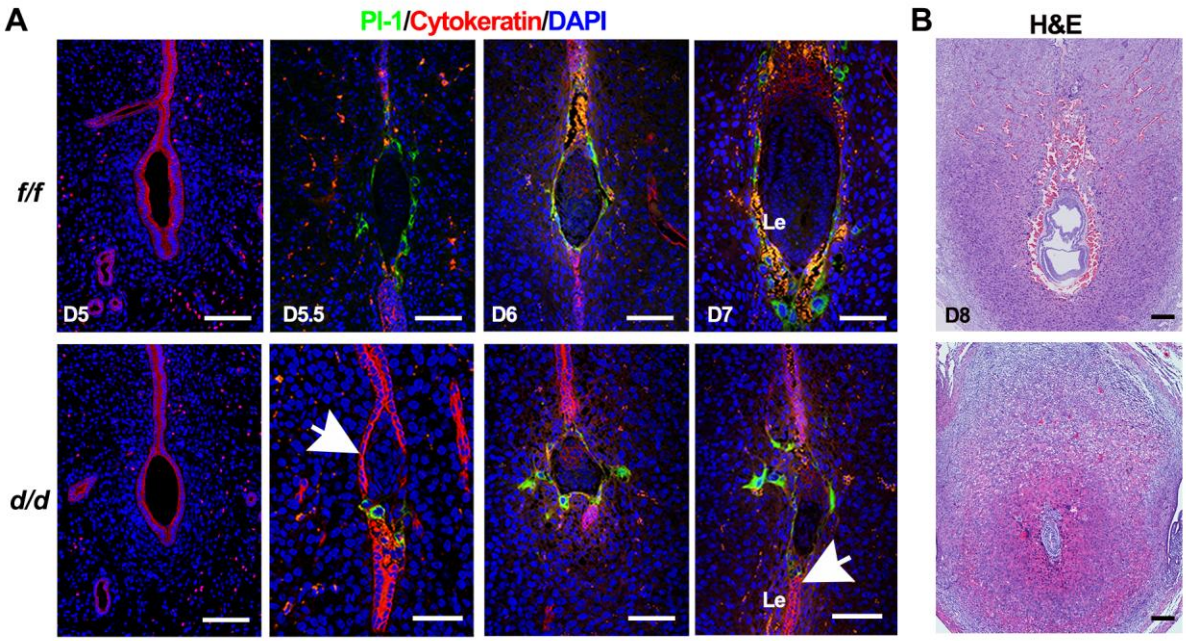
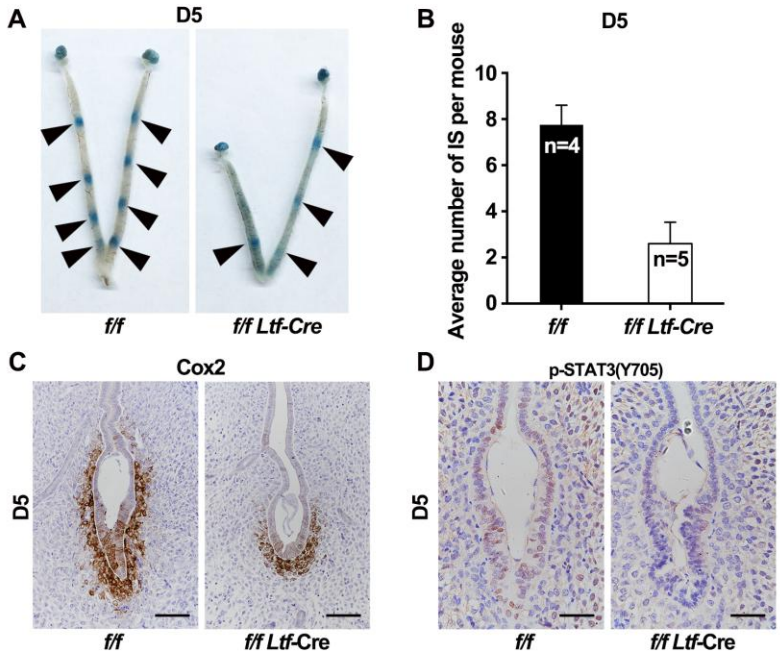
Supplementary material

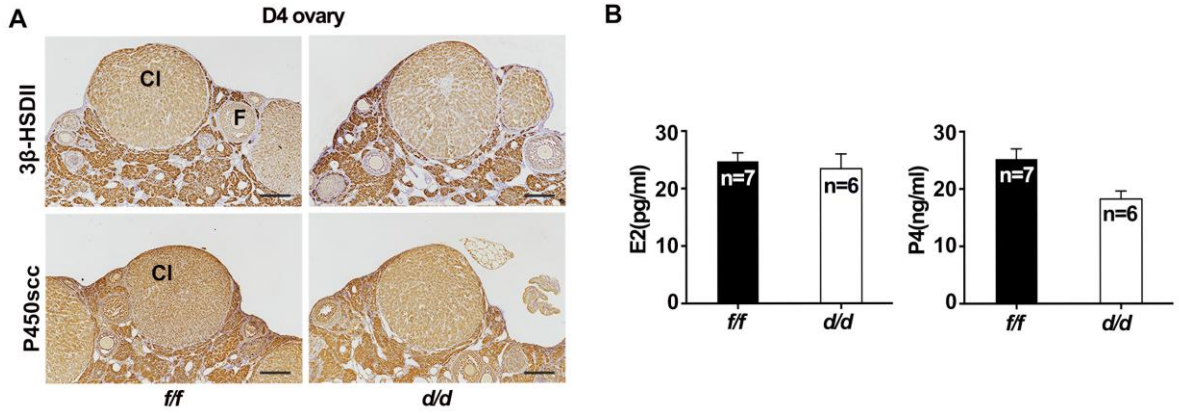
Sequential activation of uterine epithelial IGF1R by stromal IGF1 and embryonic IGF2 directs normal uterine preparation for embryo implantation



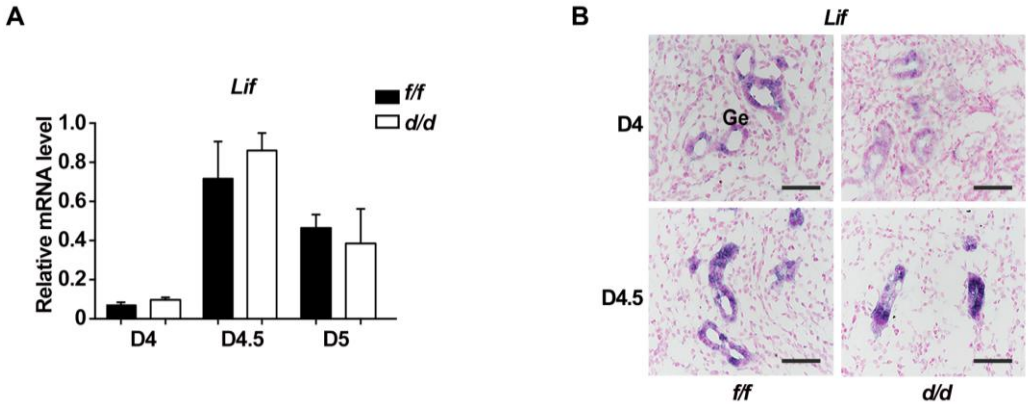
Supplementary Figure S1. Post-implantation embryo development in control and knockout mice. (**A**) Cytokeratin and Pl-1 were used to mark uterine epithelium and the outermost layer trophoblast of fetus separately from D5-D7 of pregnancy, and the fluorescent results indicated developmental retardation of a few embryos in KO uterus. (**B**) HE staining of D8 implantation sites of uteri, and the embryos had stopped growth and been absorbed. Scale bars, 50 μm. Le, luminal epithelium; the arrow indicted the persistent epithelium in the implantation chamber surrounding the embryos.



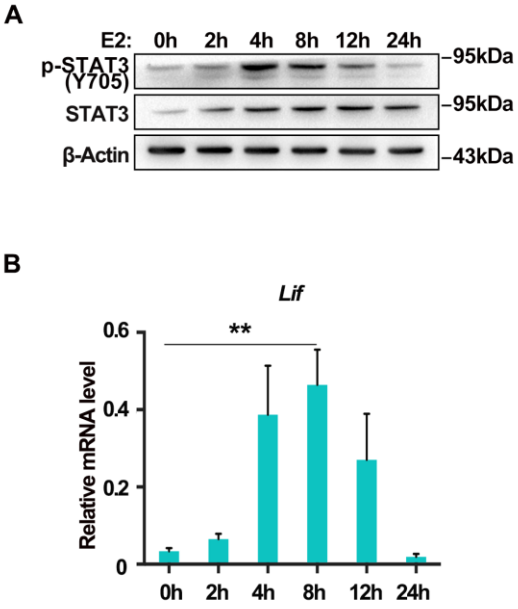
Supplementary Figure S2. Uterine epithelial-specific deletion of IGF1R impaired embryo implantation. (**A** and **B**) The average number of implantation sites (IS) showed an apparent decrease in *Igf1r*$^{f/f}$*/Ltf-cre* mice on D5. (**C**) Immunohistochemical analysis of Cox2 protein in *Igf1r*$^{f/f}$ and *Igf1r*$^{f/f}$*/Ltf-cre* uteri with a blastocyst on D5 morning. scale bars, 100 µm. (**D**) Immunohistochemical analysis of pStat3 (Y705) in uteri with a blastocyst on D5 morning. Scale bars, 50 µm.



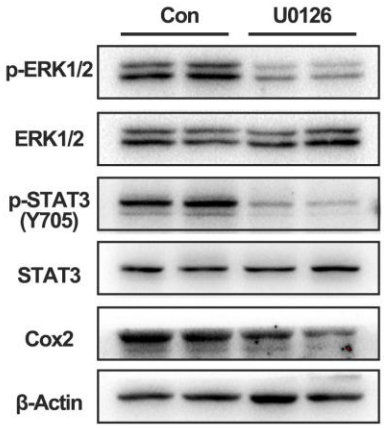
Supplementary Figure S3. Hormone levels are comparable between *Igf1r*$^{f/f}$ and *Igf1r*$^{d/d}$ mice on D4. (**A**) Immunohistochemical staining of 3β-HSDII and P450scc in D4 *Igf1r*$^{f/f}$ and *Igf1r*$^{d/d}$ ovaries. CL, corpus luteum; F, follicle. Scale bars, 200 μm. (**B**) Serum levels of E_2 and P_4 in *Igf1r*$^{f/f}$ and *Igf1r*$^{d/d}$ mice on D4 of pregnancy.



Supplementary Figure S4. The expression of LIF was comparable between *Igf1r*$^{f/f}$ and *Igf1r*$^{d/d}$ uteri. (**A**) The *Lif* mRNA level in *Igf1r*$^{f/f}$ and *Igf1r*$^{d/d}$ uteri from D4 to D5 as detected by quantitative real-time PCR. The values are normalized to the *Gapdh* expression level and indicated as the mean ± SEM, n=3. *, *P*<0.05. (**B**) *In situ* hybridization of *Lif* in *Igf1r*$^{f/f}$ and *Igf1r*$^{d/d}$ uteri on D4 and D4.5. Ge, glandular epithelium; Bl, blastocyst; S, stroma. Scale bars, 50 μm.



Supplementary Figure S5. E_2 increased the levels of STAT3 activation and Lif expression. (**A**) The expression level of p-STAT3 (Y705) and STAT3 proteins in D4 WT mouse uteri in response to E_2 treatment. Actin served as the loading control. (**B**) Quantitative real-time PCR analysis of *Lif* mRNA expression in WT mouse uteri in response to E_2 treatment. The values are normalized to the *gapdh* expression level and indicated as the mean ± SEM. n=3.



Supplementary Figure S6. U0126 treatment reduced ERK and STAT3 activation. Western blot analysis for the indicted protein in D4 uteri. The uteri were treated with IGF2 recombinase protein, combined with the control or U0126 injection for 6 hours. Actin served as the loading control.

Supplementary Table S1. Quantitative real-time PCR primers.

Primer name	Sequence
Msx1 F	CTTCCTCCTGGTTGTCGCT
Msx1 R	CTCTTGGCCTCTGCACCCTTAGTTT
Lif F	GAGAGATTTCTGTCTCACTC
Lif R	CTTCCAAAGTTTCTCTGAGA
CLDN1 F	CTGGTAGAGGTAATGTGAGT
CLDN1 R	GAGATACAGAACAGTTGAAGG
CLDN3 F	CCAGGAGAGGAGCCGTTAAG
CLDN3 R	GCTGGACCTGGGAATCAACT
CLDN4 F	CCACTCTGTCCACATTGCCT
CLDN4 R	CTTTGCACAGTCCGGGTTTG
CLDN7 F	GCTATGACTGGAGGCATT
CLDN7 R	GTGACAATCTGATGACCAATC
CLDN8 F	GCCCTCTACATAGGCTGGAC
CLDN8 R	GAAACTCCGTTGAGTGGTGC
CLDN15 F	CGTGGGCAACATGGATCTCT
CLDN15 R	CCACGAGATAGCCACCATCC
Occludin F	TGCTGCTGATGAATATAATAGAC
Occludin R	ATCCTCTTGATGTGCGATAA
Igf1r F	GCAGACACTACTACTACAAAG
Igf1r R	ACTCATCGTCGTGGATAA
Igf1 F	CGCTCTGCTTGCTCACCTTCAC
Igf1 R	AATGCTGGAGCCATAGCCTGTG
Cdh1 F	GAGACCAGTTTCCTCGTCCG
Cdh1 R	AGCAGCTCTGGGTTGGATTC
Ctnna1 F	ACGGTCTGGAGAAGGAAGGT
Ctnna1 R	ACACGAGCCCGAATAAAGCA
Ctnnb1 F	AGGATGATCCCAGCTACCGT
Ctnnb1 R	AGATCAGGCAGCCCATCAAC
Ptgs2 F	GTCTGGTGCCTGGTCTGATGAT
Ptgs2 R	GTGGTAACCGCTCAGGTGTTG
Bmp2 F	GATCTGTACCGCAGGCACTCA
Bmp2 R	AGTTCCTCCACGGCTTCTTCG

Igf2 F	TGCTGCATCGCTGCTTACGG
Igf2 R	GACGGTTGGCACGGCTTGAA
Esr1 F	TGCCTCTGGCTACCATTAT
Esr1 R	TGCCCACTTCGTAACACTT
Pgr F	ACCTGATCTAATCCTAAATGA
Pgr R	ATTGTGTTAAGAAGTAGTAAGAC
Ihh F	CATCTTCAAGGACGAGGAGAACA
Ihh R	CATGACAGAGATGGCCAGTGA
Nr2f2 F	CTTTGGAAGAGTACGTTAGG
Nr2f2 R	AACAATTGCTCTATGACTGA
Hand2 F	TCGGTTATCTAGTGCTGTC
Hand2 R	ATACTTACAATGTTTACACCTTCA
Hoxa10 F	GGCAGTTCCAAAGGCGAAAA
Hoxa10 R	CAAAAAAAGCCAGAACAAAC
Wnt7b F	TGAGGCGGGCAGAAAGG
Wnt7b R	CCTGACACACCGTGACACTTACA
Muc1 F	ATTGTGTTAAGAAGTAGTAAGAC
Muc1 R	AAGTGGTCACCACAGCTGGG
Coch F	GTGCAGCAAAACCTGCTACAA
Coch R	AGCTAGGACGTTCTCTTTGGT
GAPDH F	ATGGTGAAGGTCGGTGTGA
GAPDH R	TGAGTGGAGTCATACTGGAACAT

Supplementary Table S2. Primers for vector construct.

Primer name	Sequence
STAT3-pBiFC-VC155-F	GCCGAATTCATGGCTCAGTGGAACCAGCTG
STAT3-pBiFC-VC155-R	GAAGGTACCTCACATGGGGGAGGTAGCAC
ERK1- pBiFC-VN173-F	GAAGTCGACATGGCGGCGGCGGCGG
ERK1- pBiFC-VN173-R	GCCGGTACCTTAAGATCTGTATCCTGGCTGGAATCTAG
STAT3-Zsgreen-F	GCCGAATTCATGGCTCAGTGGAACCAGCTG
STAT3-Zsgreen-R	GAAGGTACCTCACATGGGGGAGGTAGCAC
Cox2-P1-Luciferase-F	GGGGGTACCTTCGCTACTCCATCCTCACACC
Cox2-P1-Luciferase-R	GGGAAGCTTGCTCCACTTCATCGGAATGCTA
Cox2-P2-Luciferase-F	GGGGGTACCCGCAGACTCAGCGAAC
Cox2-P2-Luciferase-R	GGGAAGCTTTTTCCGCTTAGGCTTTCC
Cox2-P3-Luciferase-F	GGGGGTACCCACCAGTACAGATGTGGACCCT
Cox2-P3-Luciferase-R	GGGAAGCTTGCTCAAGAGTGTCACAGCTTCC

Supplementary Table S3. Primers for *in situ* DIG-labelled probes.

Primer name	Sequence
Igf1r-DIG-F	GATTGATCCTAATGTATG
Igf1r-DIG-R	TTATTTCTCTTTCTATGG
Msx1-DIG-F	CCTGACTTAGGTGGGTCCAG
Msx1-DIG-R	GTCCTTTTGGCCTCTGGTCT
Igf1-DIG-F	ACAATAATAAGTCCAATAACAT
Igf1-DIG-R	GAAGAGGTGAAGATAAGG
Ptgs2 (Cox2)-DIG-F	ACTCTGCTCCGAAGAATCTC
Ptgs2 (Cox2)-DIG-R	ACATCCCTGAGAACCTGCAG
Igf2-DIG-F	ATGGGGATCCCAGTGGGGAA
Igf2-DIG-R	GTCCAGCAACCATCAGGAAT

Supplementary Table S4. ChIP–PCR primers.

Primer name	Sequence
STAT3-ChIP-P1-F	TTCGCTACTCCATCCTCACACC
STAT3-ChIP-P1-R	GCTCCACTTCATCGGAATGCTA
STAT3-ChIP-P2-F	CGCAGACTCAGCGAACCA
STAT3-ChIP-P2-R	TTTCCGCTTAGGCTTTCC
STAT3-ChIP-P3-F	CACCAGTACAGATGTGGACCCT
STAT3-ChIP-P3-R	GCTCAAGAGTGTCACAGCTTCC